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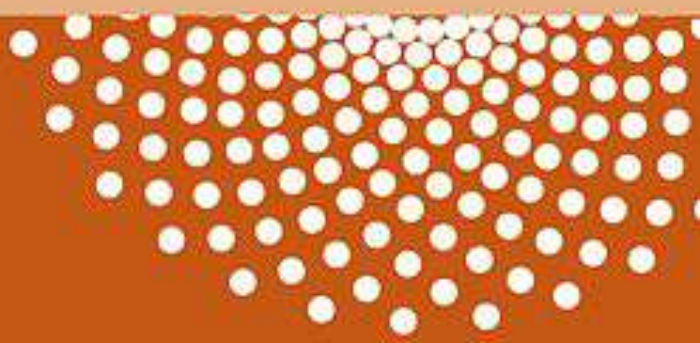

Emerging and Renewable Energy: Generation and Automation

Guest Editors:
Mohamed Becherif
Haitham S. Ramadan
Mohamed Toufik Benchouia
Mahmoud Khaled
Mohamad Ramadan
Mickael Hilairet

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Conference and Exhibition Indonesia - New and Renewable Energy and Energy Conservation (The 3rd Indo EBTKE-ConEx 2014)

PREFACE

The 3rd Indo EBTKE-ConEx 2014 is an annual event organized by the Directorate General of New and Renewable Energy and Energy Conservation (DG-EBTKE), Ministry of Energy and Mineral Resources of the Republic of Indonesia, in cooperation with the Indonesian Renewable Energy Society (IRES) and Quad Event Management. The event was held on 4–6 June, 2014 at the Jakarta Convention Center (JCC), with the theme "Time to Deliver Clean Energy for the Nation". The theme was created to revive the commitment to deliver clean energy, which is new and renewable energy and energy conservation, for the nation. It is also relevant to the Indonesian energy security and sustainability roadmap as stated in the national energy policy. Indo EBTKE-ConEx 2014 was officially opened by Prof. Dr. Boediono, Vice President of the Republic of Indonesia. This event was attended by a total of 5,009 visitors.

The 3rd Indo EBTKE-ConEx 2014 provided an excellent venue to identify opportunities in regional Indonesia that are challenging to reach. It also provided an important opportunity to share knowledge, understand challenges in renewable energy, build partnerships and strengthen cooperation among key stakeholders.

The aims of Indo EBTKE-ConEx 2014 were to:

- Increase public awareness of new and renewable energy and energy conservation ("EBTKE") concept
- Promote latest products and technology of EBTKE
- Provide platform for EBTKE key stakeholders (governments, private sectors, and academics) to network and increase collaboration to achieve Indonesia's EBTKE target
- Stimulate growth of new entrepreneurs in EBTKE as a sector, also increase the number and capacity of EBTKE projects significantly in the near future.

The conference presented papers from 40 international and Indonesian scientists, technologists, researchers, academicians, government officials, practitioners and the private sector. Presentation material from the conference can be downloaded at http://www.indoebtke-conex.com/detail.php?id=3&cat=3

The exhibition dosplayed and demonstrated various technologiest on EBTKE at 27 booths in the Jakarta Convention Center (http://www.indoebtke-conex.com/best-booth.php). Education was carried out in three ways:

- **Training on new and renewable energy,** including biogas, biomass energy, solar energy / photovoltaic, mini and small hydropower, was conducted by GIZ-Indonesia and the USAID Indonesia-Clean Energy Development (ICED Project); and training on energy efficiency was conducted by the United Kingdom Climate Change Unit (UKCCU).
- An **essay contest** on new and renewable energy and energy conservation for students from junior high schools, senior high schools and undergraduate higher education produced 123 essays (http://www.indoebtke-conex.com/writing-competition.php).
- A **scientific poster competition** on new and renewable energy and energy conservation for graduate students, researchers, lecturers / academicians, technologists, and practitioners of new and renewable, and energy conservation produced 74 posters (http://www.indoebtke-conex.com/paper-poster-competition.php).

A call for technical papers elicited 131 extended abstracts and, following a thorough peer review process, 77 manuscripts were selected for presentation in Indo EBTKE-ConEx 2014 (http://www.indoebtke-conex.com/detail.php?id=4&cat=3 **and** http://www.indoebtke-conex.com/data_proceeding.php). After selection and review by the scientific committee, 50 selected manuscripts were chosen for publication in Elsevier's Journal, *Energy Procedia*.

We wish to convey our utmost appreciation to all authors and reviewers for their high quality contributions; to all participants/visitors for their participation; and to committees for their strong support throughout the Indo EBTKE-ConEx 2014.

Peer-review under responsibility of the Scientific Committee of EBTKE ConEx 2014
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Last, but not least, we hope Indo EBTKE-ConEx 2014 provided an interesting program and served as an excellent forum for innovative and technical discussions. We look forward to seeing you all at Indo EBTKE-ConEx 2015.

Sincerely yours,

Salis Aprilian
Indo EBTKE-ConEx 2014
Chairman of Organizing Committee

Scientific Committe/Editorial & Peer Review Board Indo EBTKE-ConEx 2014

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Conference and Exhibition Indonesia - New, Renewable Energy and Energy Conservation (The 3rd Indo-EBTKE ConEx 2014)

Molecular and Histochemical Analysis of *Jatropha curcas* Linn. Transgenic Using Tolerance Antibiotics Hygromycin (*hpt*) and β-Glucuronidase (*gus-A*) Gene

Maftuchah[a,b]*, Agus Zainudin[a,b], Robby Wahyu Pahlevi[b], Arik Anggriawan[b]

[a]*Vegetable Oil Technology Laboratory , University of Muhammadiyah Malang. Jln. Raya Tlogomas 246 Malang 65144, East Java – Indonesia*
[b]*Faculty of Agriculture , University of Muhammadiyah Malang . Jln. Raya Tlogomas 246 Malang 65144 , East Java – Indonesia*

Abstract

To improve *Jatropha curcas's* trait, genetic engineering technology genetic engineering development is needed. This study was aimed to analyze transgenic of *J. curcas* with plasmid vectors pCambia-1304. The study was conducted using primer *hpt (forward)* 5'-GATGCCTCCGCTCGAAGTAGCG-3', *(reverse)* 5'-GCATCTCCCGCCGTGCAC-3', *gus-A* (*forward)* 5'–GCCATTTGAAGCCGATGTCACGCC-3' and *(reverse)* 5'-GTATCGGTGTGAGCGTCGCAGAAC-3'. The PCR results elucidated five plants (16.67 %) positive *hpt* gene, 22 plants (77.33 %) positive *gus-A* gene. Histochemical test results showed 16 plants (53.33 %) positive *hpt* test. A total of 24 plants (80 %) resulted positive *gus-A* gene on leaf and 11 plants (36.67 %) resulted positive *gus-A* gene on the root.

Peer-review under responsibility of the Scientific Committee of EBTKE ConEx 2014

Keywords: *Gus-A* gene; histochemicals test; *hpt* gene; *Jatropha curcas* Linn.

Peer-review under responsibility of the Scientific Committee of EBTKE ConEx 2014
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Nomenclature

DNA	deoksi nucleic acid
E. coli.	*Escherisia coli*
***gus-A* gene**	*β*-glucuronidase gene
hpt	hygromycin phosphotransferase
***J. curcas* L.**	*Jatropha curcas* Linn.
***nptII* gene**	neomicyn phosphotransferase gene
pCambia	plasmid from centre for application of molecular biology to international agriculture.
PCR	polymerase chain reaction
RNA	ribo nucleic acid

1. Introduction

The world's oil reserves are running low; this happens because oil is a substance that cannot be renewable, while the demand for energy continues to rise recently. Annually, the demands for energy continue to increase. However, the world's oil production continues to decrease after 2020 [1]. *Jatropha curcas* Linn. is a woody shrub plant includes in *Euphorbiaceae* family which mostly found in the tropical area. Although had been known as one of medicinal ingredients, *J. curcas* more attention as bioenergy source. *J. curcas* is considered to have prospects as biodiesel source for its high oil content, not compete for other utilization, i.e. palm oil or sugarcane, and posses interesting agronomic characteristics [2].

J. curcas is suitable to agro-climate in Indonesia, but the provision of high yielding varieties which commercially released become a problem. The plant's characters repair can be done through various ways including genetic modification, one of them is through genetic engineering technology [3]. Genetic engineering aims to obtain genetic superior cultivars by utilizing gene transfer techniques from desirable characters and eventually, it will obtain transgenic plants. Many methods are used to transfer genes into transgenic plants, one of them by utilizing bacteria as a vector carrying the gene which is inserted into the plant to alter genetic characters of plants. The insertion of genes in bacteria, which is called genetic transformation, is considered to be more effective to obtain gene expression in transgenic plants [4].

Genetic transformation technology has been developed by utilizing various methods of transformation [5]. There are two kinds of genetic transformation techniques, namely direct genetic transfer and indirect genetic transfer. Direct genetic transfer is the transfer without using any intermediary or a vector, whereas gene transfer techniques using vector indirectly. The commonly vector used is *Agribacterium tumefaciens* [6].

Genetic transformations with efficient procedures on *J. curcas* plant have successfully performed initially by [7] through *Agrobacterium tumefaciens* on cotiledone pieces. [7] used strain LBA4404 and EHA105 with *Phosphinothricin acetyltransferase* and *Hygromyicin phosphotransferase*. *Neomycin phosphotransferase II (nptII)* gene as marker selectors used explants embrio and nodal cotiledone [8]. It is then continued by the using of *Agrobacterium tumefaciens* strain LBA4404 which carry pCambia-1304 binary vector. This researched was aimed to optimize callus cultures, to obtain transgenic plants of *J. curcas*.

Gus-A gene or *β-glucuronidase* gene is a reporter gene, which aims to analyze the activity of a promoter both quantitatively and through visualization in plant's tissues, these genes derived from the *Escherichia coli* bacterium [9]. The gene expresses enzyme which catalyzing the breakdown of various compounds *glucuronidase*. This enzyme when incubated with several non-specific substrate, can transform cells into colored [10]. *Gus A* gene function is as an expression vector which shows that the desired gene has been entered into the plant, by the size of *gus-A* gene is 439 bp [11].

Hygromycin is an antibiotic produced by *Streptomyces hygroscopicus.* This antibiotic inhibits protein synthesis by disrupting translocation and cause a wrong translation in the 70S ribosome. Resistance to hygromycin is given by *hpt* gene of *E. coli.* [12]. *Hygromycin phosphotransferase test (hpt)* is a selector agent widely which widely used in plants for the selection of bacterial resistance vectors of gene carrier in genetic transformation techniques [13,14].

The resulted transgenic plants have to do a variety of tests to prove that the transformed gene has been integrated in the plants [15]. The fastest and the best analysis to indicate whether genes stably integrated in the plant is the molecular analysis of DNA through PCR and histochemical tests such as using *hpt* and *β-glucuronidase* test [16]

This study aimed to analyze *J. curcas* transgenic plant, which was resulted from genetic transformation with a plasmid vector pCambia-1304 which contains a tolerance to the antibiotic marker genes hygromicyn *(hpt)* and *gus-A* genes through molecular analysis and histochemical tests.

2. Material and method

This research was conducted at the Laboratory of Molecular, Center for Biotechnology Development, University of Muhammadiyah Malang. The research activities were carried out by using samples of 30 transgenic plants. The transgenic plants were resulted from pCambia-1304 plasmid introduction (Figure 1) which directly into the ovary of *J. curcas* plant.

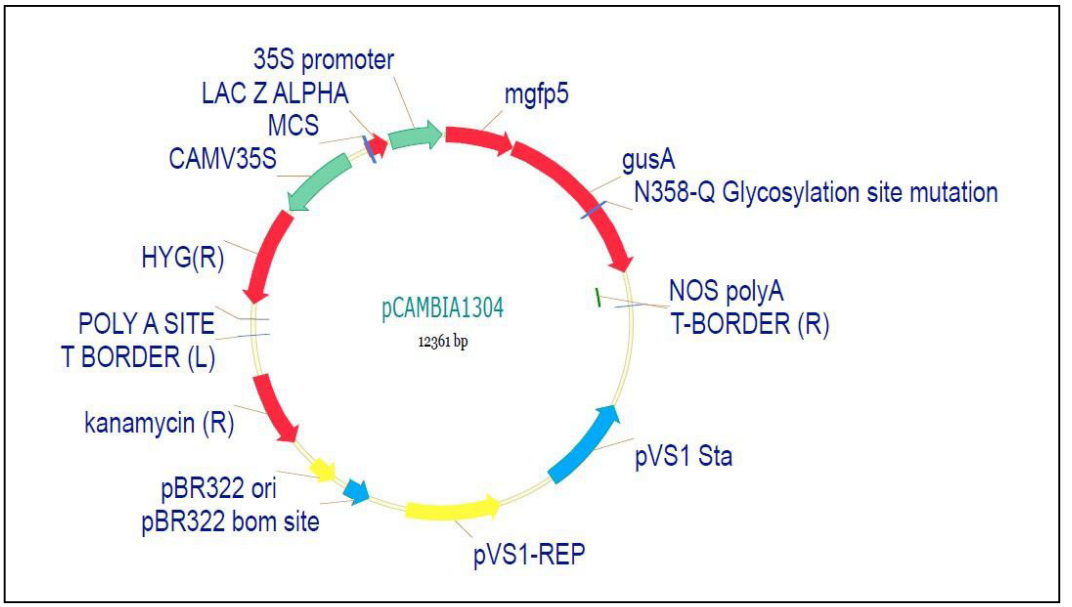


Fig. 1. Vector plasmid pCambia-1304 [2]

2.1. Analysis of PCR gus A gene and hpt gene

Molecular analysis through polymerase chain reaction (PCR) were performed by using primer *hpt (forward)* 5'-GATGCCTCCGCTCGAAGTAGCG-3', *hpt (reverse)* 5'-GCATCTCCCGCCGTGCAC-3', primer *gus-A* (*forward)* 5'-GCCATTTGAAGCCGATGTCAC GCC-3' dan *gus- A (reverse)* 5'-GTATCGGTGTGAGCGTCGCAGAAC-3'.

DNA isolation was carried by a modified CTAB method [17]. Young leaves of *J. curcas* plants were crushed with enough liquid nitrogen by using a mortar. It is then added with 200 µl of buffer extraction and put into a 1.5 ml tube. Next, add 600 µl of buffer extraction and knock it until the mix is homogeneous. Mix the disentrifuse solvent at the speed of 12 000 rpm, 4 ºC temperature for 10 min. After that, add 500 µl of lysis buffer, spin it until homogeneous. Incubate it at a temperature of 65 ºC for 30 min, and invert every 10 min. Add 500 µl of chloroform: isoamyl (24:1), then invert. Centrifuge at 12 000 rpm, 4 ºC for 10 min. Take the upper solvent and put into a new tube and then add 800 µl of absolute EtOH and 80 µl of NaOAc, invert them. Centrifuge again at 12 000 rpm, 4 ºC for 10 min, then discard the supernatant. Add another 800 µl of 70 % EtOH at deposition, and then invert them. Centrifuge at a speed of 12 000 rpm, 4 ^{o}C for 10 min, then discard the supernatant. DNA pellet were dried for ± 24 h, after dried, dissolve the DNA into 30 µl TE buffer. DNA stored in the refrigerator.

hpt gene PCR process: it was done with the initial denaturation (95 ^{o}C for 1 min), denaturation (95 ^{o}C for 1 min), annealing (70 ^{o}C for 1 min), synthesis (72 ^{o}C for 1 min), and post PCR (72 ^{o}C for 5 min). The storage of PCR results can be performed at a temperature of 4 ^{o}C [19]. Then, the gel was identified by using a UV transluminator by using gel document.

The process of *gus-A* gene PCR: it was done with the pre-denaturation (94 ^{o}C, 1 min), denaturation (94 ^{o}C, 1 min), annealing (48 ^{o}C, 1 min), synthesis (72 ^{o}C, 30 s) and post-PCR (72 ^{o}C, 5 min). Once the PCR process is complete, PCR tube containing the PCR solution is subsequently issued and stored at 4 ^{o}C temperature if it is not running directly. The resulted product of PCR is shown by the size of 492 bp at electrophoresis process.

2.2. Histochemical analysis for hpt marker gene

The materials required for histochemical tolerance analysis to higromysin antibiotics are: hygromicyn antibiotics, triton-X, gelatine, permanent markers, and sterile distilled water. Chemical preparations for *hpt* histochemical test were conducted by dissolving 20 µl hygromicyn antibiotics (M = 527.53 g · mol^{-1}) into 20 µl of sterile distilled water (hygromycin 50 %). Then, dissolve 1 µl of triton-X (M = 646.85 g · mol^{-1}) into 100 ml of sterile distilled water (triton-X 0.001 %). 250 mg of gelatin into 25 ml of sterile distilled water (1 % gelatin), and heated in a waterbath until it is boiled, then wait until the temperature decreased to 30 ^{o}C. Mix 20 µl of hygromycin 50 %, 200 µl, 0.001 % triton-X, 1 000 µl of 1 % gelatin with 780 µl sterile distilled water [18].

For the implementation of the *hpt* test, make a circle using the markers on the sample of *J. curcas* plant's leaves. Take 2.5 µl of *hpt* test solution for a single hatching. Squirt *hpt* test solution to 30 numbers of *J. curcas* transgenic plants (where each plant is treated two sheets of leaf samples and dropped the solvent right in the circle markers). Microscopic observations performed on the color change and the appearance of blue patches on leaf samples in three days after hatching. Histochemical analysis for *hpt* gene marker were done using hygromycin antibiotics, where positive *hpt* gene plants (transgenic plants) will not experience spotting on leaves, and non-transgenic plants (does not contain *hpt* marker gene) will show spots on the leaves by giving them hygromycin antibiotics [3].

2.3. Histochemical analysis of gus-A gene marker.

Materials required for histochemical analysis of *gus-A* gene marker were $NaPO_4$ buffer, triton-X, K_3Fe, X-gluc, Na_2EDTA and alcohol. The test of gus leaves and roots were done using the method of Jefferson [19] which then modified [17]. Histochemical test were conducted to determine the reaction of the protein resulting from gene markers with its substrate. First, *J. curcas*'s leaves and roots were used as the gus test material. The leaves and roots were soaked in buffer of Na-Phophat 50 nM which then added 1 % triton X-100 at a temperature of 37 ^{0}C for 1 h (in a shaker incubator). Then, they were washed by fresh buffer fosfat pH 6.8 and vacuumed for 5 min. Next, phosphate buffer was discarded. Soak the leaves into phosphate buffer solution (containing K_3Fe, X-gluc, and Na_2EDTA) with pH 6.8, and then add 5 µl of X-gluc. Vacuum for 5 min ; incubate overnight at a temperature of 37 ^{o}C to 38 ^{o}C. Observations were done with a microscope; the cells that express *β-glucuronidase* will expose blue color. If it have shown blue color, phosphate buffer was removed and replaced by alcohol 70 %.

3. Results and discussion

Nowadays, the method of genetic transformation is growing as the discovery of several researches that support the biotechnology techniques. The presence of biotechnology provides new perspective for plant breeders to obtain a new and wider gene group with certain superior characteristics [4]. Through biotechnology techniques such as genetic engineering, plant breeders will be able to produce transgenic plants which posse new characteristics such as resistance to pests, insects, herbicides, or improving the quality of results [20]. Transgenic plants that have been assembled by the entry of genes into cells which tissues' have been transformed can be confirmed by using molecular analysis through polymerase chain reaction technique [21]. Genomic DNA was isolated from plant's tissue taken from young leaves of transgenic plants and control plants [22]. The next stage, PCR test was run by using pair of primary DNA which specifically designed to amplify DNA fragments with particular gene construction [21].

The result of the PCR analysis using primer hpt gene is shown in Figure 2. Figure 2 showed the results of DNA bands expression resulting from PCR process sized 920 bp which is *hpt* gene size. Table 1 showed that from 30 samples of *J. curcas* plant leaves tested, only five plants (16.6 %) that contribute positive results of *hpt* gene.

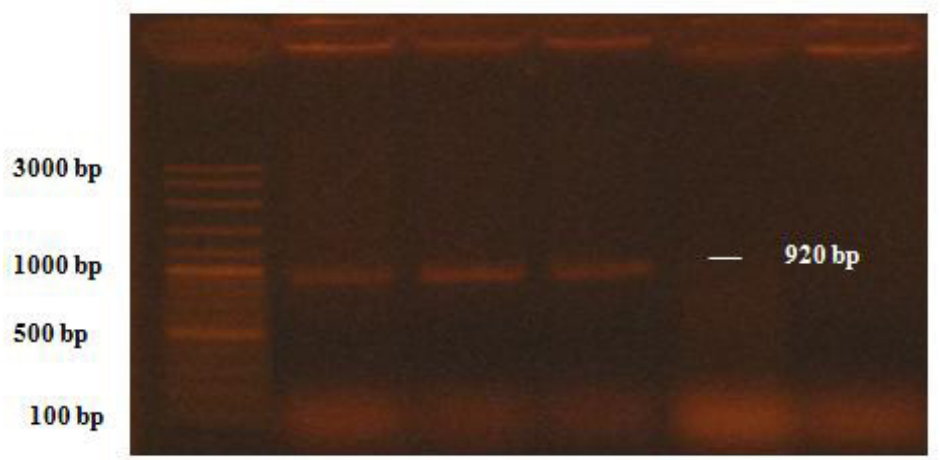


Fig. 2. PCR analysis with *hpt* gene primer (920 bp)

Figure 3 shows electrofogesis gell agarose result from expression of 492 bp sized DNA bands as a result of PCR test using primer *gus A* gene. Table 2 shows that there are 22 plants (77.33 %) which positively PCR *gus A* gene (492 bp size) of 30 *J. curcas* plants tested. The results of histochemical analysis showed that 16 plants (53.33 %) produce positive expression on hpt histochemical test (Table 1).

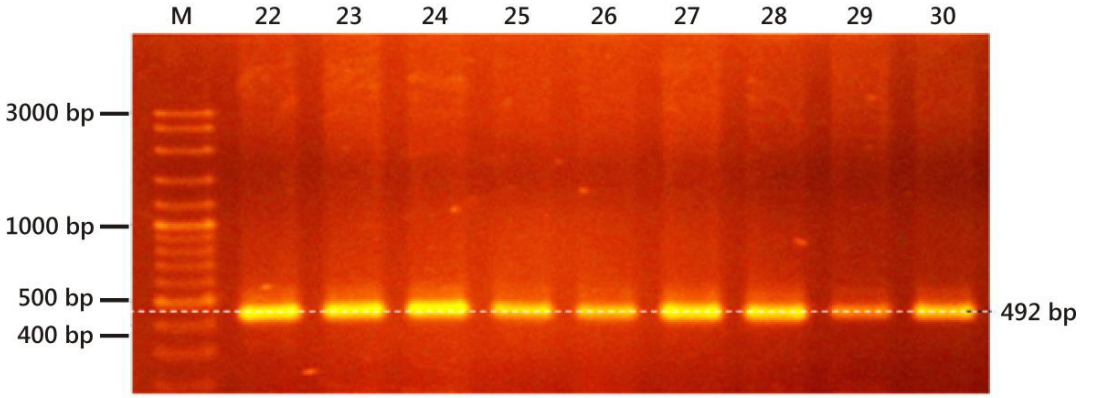


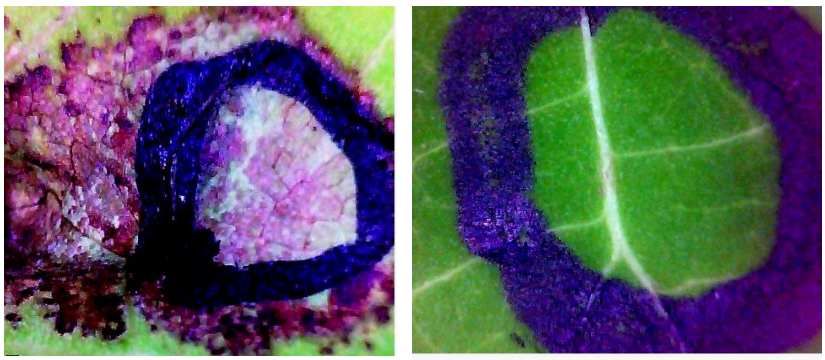
Fig. 3. *PCR* analysis with *gus-A* gene primer (492 bp)

Hygromycin tolerance test in Jatropha can be demonstrated by the absence of necrotic leaf samples of plants that spilled by hygromycin antibiotic (Figure 4). The presence of necrotic on the leaves of control plants (non-transgenic) showed plants'insecurity on the hygromycin antibiotic. By the presence of *hpt* gene in transgenic plants, the plants become resistant to the hygromycin antibiotic (Figure 4).

Histochemical analysis is one of the branches of histology science that studies about the tissue changes in the composition of living things. Histochemical is useful to study physiological functions of cells through color changes testing in the tissues of living beings. Histochemical analysis for genetic transformation testing in plants utilized the characters of plant resistance to an antibiotic. The use of hygromycin antibiotic in histochemical analysis of transgenic plants will provide a color change expression or generate necrotic spots on plant's tissue [15]. The change color is due to the chemical reactions of enzymes hygromycin fosfotranferase produced by *hpt* gene with a hygromycin antibiotic [23].

Table 1. PCR Analysis with *hpt* gene primer and hpt histochemical test

Number of plants	*hpt* PCR test	*hpt* histochemicals test	Number of plants	*hpt* PCR test	*hpt* histochemicals test
TR-1	-	-	TR-16	-	-
TR-2	-	+	TR-17	-	+
TR-3	-	+	TR-18	-	+
TR-4	-	+	TR-19	+	-
TR-5	-	+	TR-20	+	-
TR-6	-	+	TR-21	+	-
TR-7	-	+	TR-22	+	-
TR-8	-	+	TR-23	+	-
TR-9	-	+	TR-24	-	-
TR-10	-	+	TR-25	-	-
TR-11	-	+	TR-26	-	-
TR-12	-	+	TR-27	-	-
TR-13	-	+	TR-28	-	-
TR-14	-	+	TR-29	-	-
TR-15	-	+	TR-30	-	-



(a) (b)

Fig. 4. Hygromycin tolerance test in Jatropha's leaf: (a) non transgenic leaf; (b) transgenic leaf

Overall, the results of histochemical tests using *gus-A* gene showed that 24 plants (80 %) indicates positive *gus-A* gene test in the leaves of plants, and 11 plants (36.67 %) indicates positive histochemical *gus-A* gene test on the sample of plant roots (Table 2). This is indicated by the blue color expression in the roots or leaves of plants were tested (Figure 5).

Plasmids can replicate autonomously and can be found in bacterial cells, where in one cell can be found more than one plasmid with various size [24]. In general, plasmid encodes genes needed to survive in unfavorable circumstances so when the environment returns to normal, plasmid DNA can be discarded. Today, plasmid has been produced commercially by a number of companies; one of them is Cambia Australia, to be used as a cloning vector. Plasmid must have some criterias, which are small sized, have relatively high copy number, has a selection marker gene and a reporter gene, as well as having the enzyme cutting sites restriction to facilitate insertion of DNA into a plasmid vector [25].

Plasmid pCambia-1304 (Figure 1) is a vector produced by the Centre for Application of Molecular Biology to International Agriculture, Australia. This plasmid contains *hygromicin* (*hptII*), *kanamycin* (*kan*) and *gus-A* genes. This plasmid becomes a non-catalytic protein which has more sensitive detection with fluorescent protein [19].

Table 2. PCR Analysis with *gus-A* gene primer and *gus-A* histochemical test

Number of plants	gus-A PCR test	Gus-A histochemicals test	
		Leaf	Root
TR-1	+	+	-
TR-2	+	+	-
TR-3	+	+	+
TR-4	+	+	-
TR-5	+	+	-
TR-6	-	-	-
TR-7	+	-	-
TR-8	+	+	-
TR-9	-	+	-
TR-10	-	+	-
TR-11	+	+	-
TR-12	+	+	+
TR-13	+	+	+
TR-14	+	+	-
TR-15	-	+	+
TR-16	+	+	-
TR-17	-	+	+
TR-18	-	+	+
TR-19	+	+	+
TR-20	+	+	-
TR-21	+	+	+
TR-22	+	-	-
TR-23	+	+	-
TR-24	+	-	-
TR-25	-	+	-
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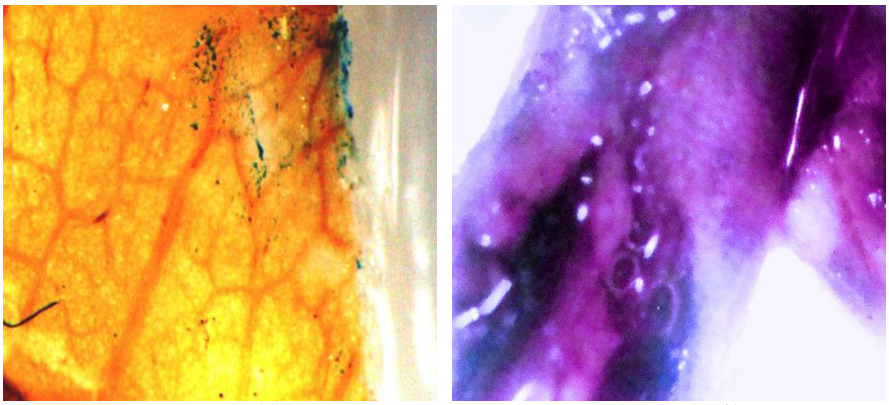
TR-27	+	+	+
TR-28	+	-	+
TR-29	+	+	+
TR-30	+	-	-



(a) (b)

Fig.5. *gus*-A test result in *Jatropha curcas* (a) leaf; (b) root

Histochemical gus test is firstly introduced by Jefferson [11]. Each enzyme test including gus, should follow these steps to avoid negative outcomes. When testing the activity of the enzyme, there are three stages to go through, they are preparation of the object, incubation with substrate and post incubation [26]. X-gluc (5-bromo-4-cloro-3-indolyl β-D-glucuronide) is a solvent that produces a blue color. With the presence of gus enzyme, the use of X-gluc histochemical analysis substrate, will give a blue color reaction that can easily be seen under a microscope. In

addition, cell types or specific tissues which express promoter gus fusion will be observed [5]. With such techniques, the activity of a specific regulatory element in plant tissues [5]. X-gluc has proven to be a good substrate for *gus* gene, which resulted in a dark blue color. First reaction resulted monomers that rapidly oxidizes to form a dimer, which is diclorodibromoindigo (ClBr-indigo) [27].

Hygromycin is an antibiotic produced by *Streptomyces hygroscopicus.* This antibiotic inhibits protein synthesis by interfering the translocation and cause mistranslation in the 70S ribosome. Resistance to hygromycin is given by the *hpt* gene of *E. coli*. [12]. *Hygromycin phosphotransferase test* (*hpt*) is an agent selector that is widely used on plants for selecting the resistance of bacterial gene vectors in genetic transformation techniques [14]. Maftuchah [5] had obtained transgenic plantlets in *Phalaenopsis* orchids. Cells' transformation in the *Phalaenopsis sp.* suspension culture using the *Agrobacterium tumefaciens* LBA4404 (pTOK233) and EHA 101 (pIG121Hm) that carry *β-glucuronidase* genes (*gus-A*) as well as resistance gene to the hygromycin antibiotic (*hpt* gene).

In transgenic rice, concentration used in *hpt* test 50 mg · L^{-1} hygromycin or 5 mg · L^{-1} phosphinothricin, however sometimes at this concentration there is still possibility for plants to escape, which is hygromycin or phosphinothricin-resistant plants but it does not contain target genes. Commonly used carbenicilin and cefotaxim antibiotics to eliminate *Agrobacterium sp*. However, carbenicilin is sensitive to the β-lakta-mase enzyme produced by bacteria so it is less effective in eliminating *Agrobacterium sp.* after co-cultivation, in contrast, cefotaxime is more resistant to β-lactamase, but can inhibit the growth of callus, plant regeneration and influence the transformation efficiency [14].

The position of genetic integration transformation in plant genomes carried by plasmids vector be cutt off or empty due to insertion gene that goes into cytoplasm are destroyed enzymatically [28]. This gene insertion if it is integrated in the heterochromatin nucleus cell area which recognized by the methyltransferase enzyme or other guardian enzymes, the gene will then be cut off so the *hpt* gene carried by the plasmid vector pCambia-1304 can not work actively.

The testing results that shows that the sample which is positively posse PCR *gus-A* gene is not necessarily posse positive *hpt* gene PCR (see Table 1 and 2). Similarly, samples with positive *gus-A* PCR is not necessarily positive in the histochemical test of leaves and roots (Table 2). The testing of genes transfer process into target plant with the plasmid vectors in a certain condition can express a failure. The unexpressed genes into the target plants in testing process of genetic transformation plants results could be due to the possibility that the T-DNA sequences are not fully inserted into the plant genome so that the gene becomes cutt off and not expressed. To see that the gene target is stable transformed, it requires gene integration patterns analysis, one of them by PCR (Polymerase Chain Reaction) technique. If the gene had been entered into the plant genome, then it will demonstrate the positive integration symptoms, but the integration analysis and gene expression analysis are still nedded in subsequent generations, so that the gene proven its well function on the elder and its generation [18]. Gene expression is a translating process series of genetic information in the sequence form of bases in DNA or RNA into proteins, and furthermore will be replicated into phenotypic expression [4].

The unintegrated marker gene on the polymerase chain reaction process of plants' genetic transformation results could be due to there is possibility that the T-DNA sequences are not fully inserted into the plant genome so that the target gene (*hpt*) be cut off or it is not inserted into the plant genome, so the possibility numbers of jatropha which indicate negative *hpt* marker gene in the plant genome with target genes that are empty or cut off in the DNA sequence. This phenomenon is referred to as gene silencing that occurs in transcription phase and post-transcriptional after gene transformation processes [22]. Silencing at transcriptional gene stage is identified by the absence its' gene transcripts and occurs when there is homology between the promoter sequences with target genes. While the post-transcriptional gene silencing occurs because the gene contains homologous sequences, so although the gene is actively transcribed but the stable mRNA that is formed only slightly [28].

Transgenic plants containing *hpt* gene which had been given treatment of solvent test, then it will not show necrotic symptoms or slightly show necrotic symptoms (tolerant) when compared to control plants or non-transgenic plants (Figure 4). This is due to the transgenic plants, hygromycin fosfotranferase enzyme produced by *hpt* gene detoxifies aminosiklitol hygromycin and catalyze the phosphorylation of hydroxyl compounds in hygromycin antibiotic, so there will be phosphate groups additional to proteins or organic molecules hygromycin. The phosphorylation process can improve the catalytic efficiency of the enzyme, converting it into active form in a protein, while other phosphorylation enzymes will convert it into an inactive form and finally the hygromycin antibiotic will work inactive poisoning plants so it will not showed necrotic symptoms on the leaves of transgenic plants [18].

4. Conclusion

The results of this reseach indicated that on molecular test through polymerase chain reaction, there are five plants (16.67 %) positive *hpt* gene and 22 plants (77.33 %) positive *gus-A* gene. The histochemical test showed that 16 plants (53.33 %) positive *hpt* histochemistry test. Overall, 24 plants (80 %) resulted in positive expression of *gus-A* gene test on *J. curcas* plant's leaves and 11 plants (36.67 %) resulted in a positive *gus-A* gene on histochemical test in *J. curcas* plant's roots.

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